#18
12/31/01

PATENT
Attorney Dock t No. 8076.102USC1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)	
HADDADA, Hedi et al)	Group Art Unit: 1633
Serial No.: 09/204,427)	Examiner: M. Wilson
Filed: December 3, 1998)	

For: DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMUROL TREATMENT

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Philippe Slos do hereby declare the following:

1.) That I have received a Ph.D. Thesis from the UNIVERSITY LOUIS-PASTEUR in MOLECULAR BIOLOGY.

2.) That I have worked as Project Leader for TRANSGENE S.A. for 10 years. Enclosed, please find a copy of my *Curriculum vitae*.

3.) That I have read the claims being submitted with the refiling of the above-mentioned patent application. Claim 1, for instance, is directed to a method of for treating a tumor in a patient by injecting an effective amount of a pharmaceutical composition into the tumor. The pharmaceutical composition is a defective adenoviral vector containing a nucleic acid insert coding for a cytokine. The defective adenoviral vector lacks the transactivators E1A and E1B, as well as the E3 regi n. Furthermore, th

defective adenoviral vector comprises a set of essential sequences needed for encapsidation.

4.) It is my opinion that the therapeutic treatment encompassed by the claims of the above-captioned patent application are in fact reproducible and patients receiving the defective adenoviral vector would see at least a decrease in tumor growth. I came to this conclusion for the following reasons.

5.) It is well known that in pre-clinical trials that regression of various tumors was achieved in mice using defective adenoviral vectors having a nucleic acid coding for a cytokine. For instance, in Bramson et al (Exhibit 1) it was demonstrated that when mice bearing breast tumors were treated with a single dose of a defective adenoviral vector expressing interleukin-12, a regression of the tumor occurred in greater than 75% of the treated tumors. The adenoviral vector in this instance was injected intratumorally and approximately one third of the mice remained tumor free. Moreover, very high levels of production of localized cytokine was confirmed.

6.) Similarly, Zhang et al (Exhibit 2) used an adenoviral vector containing an interferon gene for treating a human breast cancer cell line (MDA-MB-435) in mice and such treatment resulted in tumor regression in 100% of the animals.

7.) Cordier et al (Exhibit 3) demonstrated that complete disappearance of P815 murine mastocytoma tumors in up to 75% of the cases occurred when a defective adenoviral vector (having deletions in the E1 and E3 region) containing the murine IL-2 gene was injected into tumor cells in mice. Furthermore, the successfully treated mice developed a long lasting state of immunity during which further challenges with the tumor cells were rejected.

8.) Gambotto et al (Exhibit 4) tested whether a defective ad noviral vector containing interl ukin-12 could regress a subcutane us MC38 murine

ad nocarcinoma and a MCA205 murin fibrosarcoma. Complete regression of thes tumors was bserved, as well as the demonstrati n of the induction of long-lasting antitumor immunity.

9.) Huang et al (Exhibit 5) teach the use of a recombinant adenoviral vector expressing murine interleukin-2 to treat hepatocellular carcinoma in mice. The surviving animals also developed systemic antitumoral cellular immunity that protected them against challenges of parental hepatoma cells implanted at distant sites.

10.) Toloza et al (Exhibit 6) disclose E1 deleted adenoviral vectors encoding human interleukin-2 which may be useful in generating tumor vaccines *ex vivo*. High transient cytokine expression levels were achieved for twelve (12) different human melanomas, 2 murine fibrosarcomas and eight (8) other tumor cell lines. Most of the cell lines exhibited 100% transduction efficiencies.

11.) All of the above documents illustrate that in pre-clinical trials defective adenoviral vectors encoding various cytokines can be used to treat various tumors. Thus, the successful treatment of a variety of tumors in mice is indicative of the fact that treatment may be successful in humans.

12.) In fact, Phase I clinical trials using a defective adenoviral vector with a lacZ marker were undertaken as described by Tursz et al (Exhibit 7). The conclusions reached in this Phase I trial is set forth below:

> This ongoing phase I study has demonstrated that a recombinant adenovirus-mediated marker gene, such as rAD.RSV beta-gal, can be safely introduced into humans and that the gene is expressed by lung tumor cells of the host.

Thus, Tursz et al demonstrated that recombinant defective adenoviral vectors are safe to use in human subjects.

13.) Kendra et al (Exhibit 8) disclose the use of a d fective adenoviral v ctor which expresses Interferon-γ for use in Phase I clinical trials to treat patients with malignant melanoma. Although the E4 region was deleted as well as the E1 and E3 regions in the vector, the results appear to be promising.

14.) Leroy et al (Exhibit 9) is a review article concerning cancer immunotherapy by direct gene transfer *in vivo* of immunomodulatory genes. This review article concludes that in animal experiments with both spontaneous metastatic and non-metastatic cancers a direct *in vivo* transfer of immunomodulatory genes such as cytokines can prevent tumor growth or significantly delay the relapse of naturally occurring tumors. This publication also discloses that various clinical trials are ongoing.

15.) Stewart et al (Exhibit 10) reviews the results of a Phase I clinical trial using a defective recombinant adenoviral vector in which the E1 and E3 regions were deleted. This adenoviral vector expressed interleukin-2. The results of this trial are set forth at page 357 wherein the following was stated:

> The results of this trial encourage further exploration of AdCAIL-2 and other adenovector delivered cytokines in clinical tumor immunotherapy.

In fact, tumor regression was observed after injection of the AdCAIL-2 vector in 24% of the patients in this trial.

16.) Thus, clinical trials, as well as pre-clinical trials all lead to the fact that treatment of various tumors with defective adenoviral vectors expressing a cytokine is safe, reproducible and feasible.

17.) I further declare that all statements mad herein of my knowledg are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

23th October 2001 — *Date*

Philippe SLOS

SLOS PHILIPPE, PH.D.
PROJECT DIRECTOR

Home address
137, rue de la Schwang
67340 Weinbourg
Born 30 june 1962
Nationality : Belgium
Telephone : 33 (0) 3 88 89 26 59
Email : philippe.slos@oreka.com
philippe.slos@libertysurf.fr

Office address
Transgene SA
11, rue de Molsheim
67082 Strasbourd Cedex France
Telephone : 33 (0) 88 27 91 45
Fax : 33 (0) 3 88 22 58 07
Email : slos@transgene.fr

EDUCATION

1980-1984: **Ingénieur Industriel**, Option des Industries Agricoles et Alimentaires. Institut Supérieur Industriel du Hainaut (Belgium)

1986-1990: **Ph.D. Thesis in Molecular Biology**, Université Louis-Pasteur de Strasbourg. « Development of gene transfer system and expression vectors for Steptococcus thermophilus »

PROFESSIONAL

1984-1989: **Cadre technique** at Transgène S.A. (Strasbourg). Molecular Biology of lactic acid bacteria. In vitro transcription/translation of the CFTR cDNA.

1990- 1991 **Research Scientist** at Transgène S.A.

1991-1992: **Projet leader.** Isolation of the cDNA coding for canine IFN-γ; production of the corresponding protein in a prokaryotic expression system (*E. coli*) ; purification of an active molecule and development of a biological activity test.

1992-1995: Development of an highly effective expression/secretion system for cholera toxin subunit B (Cholera vaccine) using *E. coli*-based technology. Development of mucosal vaccine strategies using recombinant fusion proteins between cholera toxin subumit B and relevant immunodominant peptide epitopes. Follow-up of two students .

1993-1995: Development of life vaccine based on lactid acid bacteria to induce mucoal immunity.

1996-2000 : Immuno-gene therapy of cancer. Preclinical studies using adenovirus-based

vectors to deliver *in situ* cDNA coding for cytokines (IL-2 and IFN-γ).

1998-2000 : **Projet leader (Immun therapy of cancer, Ad-IFN-γ)**

2001- : **Project Direct r (Immunotherapy of cancer, Ad-IFN-γ, Ad-IL-2, Adenovirus Industrial Process)**

PUBLICATIONS.

1. C. Vander Wauven, **P. Slos** and V. Stalon. 1984. Enzymes of oxalurate utilization and the control of their synthesis in *Streptococcus faecalis*. Arch. Int. Physiol. Biochim. **92**, B68-B69.

2. C. Vander Wauven, J.P. Simon, **P. Slos** and V. Stalon. 1986. Control of enzyme synthesis in the oxalurate catabolic pathway of *Streptococcus faecalis* : evidence for the existence of a third carbamate kinase. Archives Microbiol.**145**, 386-390.

3. D.A. Romero, **P. Slos**, C. Robert, I. Castellino and A. Mercenier. 1987. Conjugative mobization as an alternative vector delivery system for lactic Streptococci. Appl. Environ. Microbiol. **53**, 2405-2413.

4. A. Mercenier, C. Robert, D.A. Romero, **P. Slos** and Y. Lemoine. 1987. Transfection of *Streptococcus thermophilus* spheroplasts, *in* Streptococcal Genetics, J.J. Ferretti and R. Curtiss III eds., American Society for Microbiology, Washington, D.C.

5. A. Mercenier, **P. Slos**, M. Faelen and J.P. Lecocq. 1988. Plasmid transduction in *Streptococcus thermophilus*. Mol. Gen. Genet. **212**, 386-389.

6. B. Boizet, D.Villeval, **P. Slos**, M. Novel, G. Novel and A. Mercenier. 1988. Isolation and structural analysis of the phospho-β-galactosidase from *Streptococcus lactis* Z268. Gene. **62**, 249-261.

7. A. Mercenier, C. Robert, D.A. Romero, I. Castellino, **P. Slos** and Y. Lemoine. 1988. Development of an efficient spheroplast transformation procedure for *S. thermophilus*: the use of transfection to define a regeneration medium. Biochimie. **70**, 567-577.

8. L. Benbadis, M. Faelen, **P. Slos**, A. Fazel and A. Mercenier. 1990. Characterization and comparison of virulent bacteriophages of *Streptococcus thermophilus* isolated from jogurt. Biochimie. **72**, 855-862.

9. **P. Slos**, J.C. Bourquin, Y. Lemoine and A. Mercenier. 1991. Isolation and characterization of chromosomal promoters of *Streptococcus thermophilus* subsp. *thermophilus*. Appl. Environ. Microbiol. **57**, 1333-1339.

10. W. Daelemans, J. Hinnrasky, **P. Slos**, D. Dreyer, C. Fuchey, A. Pavirani and E. Puchelle.1992. Immunocytochemical analysis reveals differences between the subcellular localization of normal and ΔPhe508 recombinant cystic fibrosis transmembrane conductance regulator. Experim. Cell Res. **201**, 235-240.

11. **P. Slos**, D. Speck, N. Accart, H.V.J. Kolbe, D. Schubnel, B. Bouchon, R. Bishoff and M.P. Kieny. 1994. Recombinant cholera toxin B subunit in *Escherichia coli*: high-level secretion, purification and characterization. Prot. Express. Purif. 5, 518-526.

12. **P. Slos**, P. Dutot, J-M Balloul and A. Mercenier. 1996. Immunogenicity of rCTB produced in *Escherichia coli* and of rCTB proteins. *In* Mucosal Immunization, Genetic approaches and Adjuvants (N. Mulford, L. Savage and C. Sussman, eds) IBC Biomedical Library, pp 1.12.1-1.12.19.

13. A. Mercenier, P. Dutot, P. Kleinpeter, M. Aguirre, P. Paris, J. Reymund and **P. Slos**. 1996. Development of lactic acid bacteria as live vectors for oral vaccines. Adv. Food. Sci. 18, 73-77.

14. P. Hols, **P. Slos**, P. Dutot, J. Reymund, P. Chabot, B. Delplace, J. Delcour and A. Mercenier. 1997. Efficient secretion of the model antigen M6::gp41E in *Lactobacillus plantarum* NCIMB8826. Microbiology. 143, 2733-2741.

15. **P. Slos**, P. Dutot, J. Reymund, P. Kleinpeter, D. Prozzi, M-P. Kieny, J. Delcour, A. Mercenier and P. Hols. 1998. Production of cholera toxin B subunit in *Lactobacillus*. FEMS Microbiology Letters. 169 : 29-36.

16. P. Leroy, **P. Slos**, H. Homman, P. Erbs, Y. Poitevin, E. Régulier, F.Q. Colonna, P. Devauchelle, C. Roth, A. Pavirani and M. Methali. 1998. Cancer immunotherapy by direct in vivo transfer of immunomodulatory genes. Res. Immunol. 149, 681-684.

17. **P. Slos**, M. De Meyer, P. Leroy, C. Rousseaux and B. Acres. 2001. Immunotherapy of established tumors in mice by intra-tumoral injection of an adenovirus vector harboring the human IL-2 cDNA : induction of CD8+ T cell immunity and NK activity. Cancer Gene Therapy. 8, 321-332.

ORAL COMMUNICATIONS

P. Slos. Immunogenicity of rCTB produced in *Escherichia coli* and of rCTB fusion proteins. IBC's Third Annual International Conference on Mucosal Immunization, Genetic approaches and Adjuvants, 16-18 october 1995, Rockville, USA.

P. Slos. Intra-tumoral delivery of Interferon-gamma cDNA with an adenoviral vector in combination with systemic chemotherapy : preclinical studies in murine models. Ninth International Conference on Gene Therapy of Cancer. December 7-9 2000. San Diego, USA.